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Versatile Thiol-Based Reactions for Micrometer- and Nanometer-Scale Photopatterning of Polymers and Biomolecules

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Abstract:

Thiol-based chemistry provides a mild and versatile tool for surface functionalization. In the present work, mercaptosilane films were patterned by utilizing UV-induced photo-oxidation of the thiol to yield sulfonate groups via contact and interferometric lithography (IL). These photo-generated sulfonic acid groups were used for selective immobilization of amino-functionalized molecules after activation with triphenylphosphine ditriflate (TPPDF). Moreover, protein-resistant

poly(oligoethyleneglycolmethacrylate) (POEGMA) brushes were grown from the intact thiol groups by a surface-induced polymerization reaction. Exploiting both reactions it is possible to couple amino-labelled nitrilotriacetic acid (NH_2-NTA) to sulfonate-functionalized regions, enabling the site-specific binding of green fluorescent protein (GFP) to regions defined lithographically, while exploiting the protein-resistant character of POEGMA brushes to prevent non-specific protein adsorption to previously masked areas. The outstanding reactivity of thiol groups paves the way towards novel strategies for the fabrication of complex protein nanopatterns beyond thiol-ene chemistry.

Introduction

Recent years have seen increasing interest in the use of thiol-based chemistry for surface conjugation in a diversity of fields in materials science and biotechnology .[1,2] This fact can mainly be explained by the "click"-like behaviour of these reactions, which enable the preparation of complex and highly functional molecules in a straightforward modular fashion. A series of thiol-based reactions, including the thiol-ene[3,4,5], thiol-yne[6], thiol-isocyanate[7], thiol-para-fluorostyrene[8] and thiol-bromo[9] processes, fulfils most of the characteristics of modular click reactions. Although many of these reactions have been known for several decades, interest in their use has exploded in the last few years, as a result of the search for alternatives to the widely-used, classical copper-catalysed azide/alkyne click chemistry introduced by Sharpless in 2001.[10] Besides the modification of flat 2D surfaces, thiol based click reactions have been successfully applied for the functionalization of 3D surfaces for example occurring in porous polymeric materials.[11]

One of the biggest challenges for click-type reactions is their applicability for the patterned immobilization of biomolecules on solid substrates. The most widely used strategies for biomolecular patterning at present rely upon surface activation strategies, e.g. based on carbodiimide chemistry and interactions between Ni-NTA and His-tagged proteins.[12,13] Also, the photoinduced thiol-ene

reaction has already been successfully applied in sub-µm scale patterning by selective biotinylation of mercapto-functionalized silicon oxide surfaces.[14] For protein immobilization, such strategies have to be combined with a suitable means to inhibit non-specific adsorption (fouling); it must be possible to control the surface activation step spatially so that attachment only occurs at the desired locations. In the absence of adequate control of non-specific adsorption a significant degradation in resolution results.

For the successful realization of biological structures with dimensions in the sub µm range, lithographic techniques must be combined with appropriate chemical immobilization strategies while at the same time ensuring control of non-specific protein adsorption. This represents a stringent set of criteria. A variety of techniques exist for the nanopatterning of surfaces, e.g. dip-pen nanolithography[15,16,17] and nanoshaving/nanografting[18,19,20], but few offer the potential to execute defined chemical reactions in this length scale required for the subsequent immobilization procedure. One approach fulfilling this requirement is the electro-oxidation of alkyl[21] or mercapto[22] groups by means of conductive atomic force microscopy. Alternatively, photochemical methods such as interferometric[23] and scanning near-field[12,24] lithography paves the way towards chemical transformations with resolutions of the order of 100 nm and, potentially, smaller. A significant advantage offered by the use of interferometric methods is that they facilitate patterning over large (square centimeter) areas, thus making them ideal for adaptation to biological applications. Moreover, this technique requires only modest resources, and it should be capable of exploitation by many laboratories not otherwise equipped with infrastructure for nanofabrication.

In the present work, mercaptopropyltrimethoxysilane (MPMS) functionalized silicon oxide surfaces have been investigated for the realization of complex bionanostructures (see schematic diagram in Figure 1). It is well known that surface bound thiol groups undergo oxidation to disulfides under ambient conditions. Subsequent oxidation to sulfinate and sulfonate derivatives occurs at a very slow rate,[25] although direct oxidation by means of electrochemistry or by a chemical treatment using a mixture of H_2O_2/HOAc is feasible.[26] Additionally, UV exposure provides a simple means to cause

spatially defined conversion of thiols to sulfonates.[27,28] A selective derivatization of the formed sulfonic acid groups may enable new approaches for surface conjugation. However, sulfonates are not accessible for carbodiimide chemistry, which is commonly used for selective surface modification of carboxylic acid groups. [29] A well reported approach for the activation of sulfonates is their conversion to the corresponding acid chloride using thionyl chloride.[30] Nevertheless, this derivatization strategy requires relatively harsh conditions ($SOCl_2$ under reflux) limiting its applicability.

Here we demonstrate that a mild and selective functionalization of photo-generated sulfonate groups is feasible, which enables the coupling of NTA, facilitating the site specific binding of green fluorescent protein (GFP). Moreover, protein-resistant poly(oligoethyleneglycolmethacrylate) (POEGMA) brushes were grown from the intact thiol groups by a surface-induced polymerization reaction.

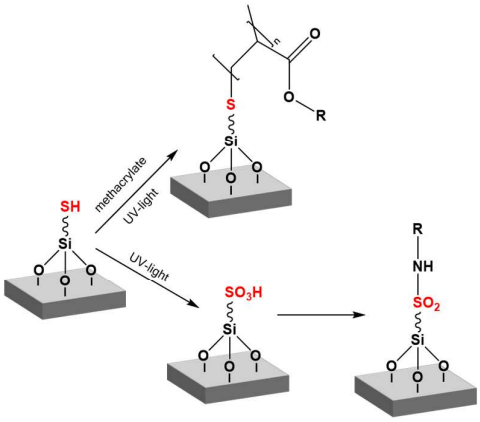

Figure 1: Overview of the thiol based surface modification reactions

The photooxidation of mercapto groups with subsequent activation and derivatization, enlarge the toolbox of thiol associated surface reactions and offer in combination with appropriate photolithographic methods novel routes for the realization of complex bionanostructures.

Experimental

Materials

Absolute ethanol (anhydrous, Sigma Aldrich), DMSO (anhydrous, Sigma Aldrich), 1,4-Dioxane (anhydrous, Sigma Aldrich), toluene (CHROMASOLV® Plus, Sigma Aldrich), pyridine (ACS reagent, Sigma Aldrich), triphenylphosphine oxide (98%, Sigma Aldrich), trifloromethanesulfonic anhydride (≥99%, Sigma Aldrich), triethylamine (puriss. p.a., Sigma Aldrich), heptadecafluoroundecylamine (≥97%, Sigma Aldrich), oligoethyleneglycol methacrylate (M_n~500 g/mol, Sigma Aldrich), oligoethyleneglycol acrylate (M_n~480 g/mol, Sigma Aldrich) were used as received.

Single-side-polished p-type-doped (B) silicon wafers with native silicon oxide (from Infineon Technologies Austria AG) were initially cleaned with piranha solution, a mixture of 30% H_2O_2 and 98% concentration sulfuric acid (both purchased from Sigma Aldrich) in the ratio 3:7 for 30 min. (Caution: Piranha solution is a strong oxidizing agent, that is known to detonate spontaneously upon contact with organic material. Handle with extreme care.)

Sample preparation

A 5 cm^2 piece of cleaned silicon oxide substrate was immersed in a solution of mercaptopropyltrimethoxysilane (MPMS, 100 µL in 20 mL toluene) for 30 min in nitrogen atmosphere. Afterwards the sample was rinsed with toluene and subsequently with ethanol/toluene (1:1 mixture) and then ethanol. After rinsing, the sample was dried under a stream of nitrogen. The samples were photo-oxidized by means of UV irradiation under ambient conditions using either a mask aligner (MJB4 SUSS, Germany) equipped with a 500 W HgXe lamp (power density of 21.3 mW/cm^2) or a coherent Innova 300C FreD frequency-doubled argon ion laser (244 nm, Coherent UK, Ely, UK) for static SIMS experiments. *(**Caution**: UV irradiation causes severe eye and skin burns. Precautions (UV protective goggles, gloves) must be taken!)*

Interferometric lithography (IL)

Films formed by the adsorption of MPMS were photopatterned by interferometric lithography (IL) using a Lloyd's mirror two-beam interferometer. A Coherent Innova 300C FreD frequency-doubled argon ion laser (Coherent UK, Ely, UK), emitting at 244 nm was used as a light source. The laser beam was focused using a lens through a spatial filter with aperture 5 μm to obtain a coherent beam. The edge of the coherent beam was cut by using a mask with an appropriate aperture. Half of the clean coherent beam was pointed directly onto the sample surface, and the other half of the beam was pointed onto the mirror, from which it was reflected onto the sample surface where it interfered with the other half of the beam to yield a sinusoidal pattern of intensity. SAMs of MPMS on silicon were exposed to 15-20 J cm^{-2} through a Lloyd's mirror interferometer, with angle 2θ between the two half of the beam set to approximately 10°.

Sample derivatization

Derivatization of sulfonic acid groups

The photo-oxidized samples were immersed in a mixture of pyridine and water (ratio 1:2) for 30 min to give the corresponding pyridine sulfonate. Afterwards the samples were dried under vacuum. The activation of the sulfonate by triphenylphosphine ditriflate (TPPDF) and the subsequent reaction with the functional amine were performed under nitrogen atmosphere. Therefore, triphenylphosphine ditriflate (TPPDF) was freshly prepared from triphenylphosphine oxide (0.019 mmol) and trifloromethanesulfonic anhydride (0.009 mmol) in dichloromethane (6 mL). The samples were immersed in a freshly prepared triphenylphosphine ditriflate (TPPDF) solution for 30 min. Subsequently, a solution of triethylamine (35 mmol) and the corresponding amine (35 mmol, heptadecafluoroundecylamine) in dichloromethane (6 mL) was added. For the derivatization with N_{α},N_{α}-Bis(carboxymethyl)-L-lysine hydrate DMSO was used as solvent. After 18 h the samples were removed from the solution and rinsed thoroughly with dichloromethane and ethanol abs.

Surface induced polymer brush formation

For the brush formation experiments, 4 µL of the monomer solution (oligoethyleneglycol methacrylate (OEGMA) in 1,4-Dioxane) was deposited on a 1 cm^2 piece of a MPMS modified silicon wafer and covered with a quartz chromium mask. Brush formation was accomplished after UV irradiation with a medium pressure Hg lamp (100 W, model 66990, Newport Corp., Irvine, California, USA) with a power density of 14.7 mW/cm^2 (measured with an EIT Power Puck II in the spectral range between 250-390 nm). Brush formation on the pre-oxidized samples was conducted with the same setup as described above using neat OEGMA and a quartz glass allowing flood illumination.

Protein immobilization

For site-specific immobilisation of His-tagged GFP, the samples were immersed in a 100 mM aqueous solution of nickel (II) chloride for 30 min. The samples were washed with water, dried with nitrogen and immersed into a 20 μg/mL solution of His-tagged GFP in phosphate buffered saline (0.01 M PBS at pH 7.4) for 24 hours at 2-4 ˚C. The samples were then further rinsed with PBS and dried with nitrogen.

Characterization techniques

The static contact angles were obtained by using the sessile drop method with a Drop Shape Analysis System DSA100 (Krüss GmbH, Hamburg, Germany).

XPS spectra were recorded using a Thermo Scientific instrument equipped with a monochromatic Al Kα X-ray source (1486.6 eV). High resolution scans of the C1s region were acquired at pass energy of 20 eV and a step size (resolution) of 0.1 eV. Scans of the S2s region were performed at pass energy of 100 eV and a step size of 0.1 eV. Wide scans were acquired with pass energy of 100 eV and a step size of 1.0 eV. All spectra have been normalized to the Au 4f7/2 peak. Charge compensation was performed with an argon flood gun. The average chemical composition was calculated from wide scan spectra in two different locations on each surface. The peaks were fitted using a Gaussian/Lorenzian mixed function employing Shirley background correction (Software Thermo Avantage v5.906). All analyses were performed at room temperature.

Static SIMS experiments were carried out using a TOF-SIMS V instrument (Ion-ToF GmbH, Münster, Germany) equipped with a Bismuth ion gun and a single-stage reflectron time-of-flight analyzer. During imaging charge neutralization was applied. A minimum of 3 images per sample acquired and multiple samples analyzed. High mass-resolution images were obtained by using high-current

bunched mode, with Bi_3^{++} as the primary source and a target current of ca. 0.1 pA. To ensure the images were static SIMS, the primary ion dose was limited to $5*10^{12}$ ions/cm^2. All data was analyzed with SurfaceLab 6 software (Ion-Tof). Images of similar ions were grouped together (e.g. S^- and SH^-) and 4 pixels were binned together.

AFM micrographs were recorded with either a Nanosurf FlexAFM instrument or Nanoscope IIIa (Bruker). Silicon AFM probes with a resonance frequency of 190 kHz and a force constant of 48 N/m (Tap190AL-G, Budgetsensors) or probes with a resonance frequency of 300 kHz and a force constant of 26 N/m (OTESPA-R3, Bruker) were used for tapping mode measurements, silicon AFM probes with a resonance frequency of 13 kHz and a force constant of 0.2 N/m (ContAL-G, Budgetsensors) for friction force measurements.

The average molecular weights (Mw and Mn) were determined by gel permeation chromatography with tetrahydrofuran (THF) as a solvent, using the following arrangement: micro-volume double pistol pump, flow rate 1 mL/min, separation columns from Varian, particle size 5 µm, combined refractive index-viscosity detector. Polystyrene standards from Polymer Labs were used for calibration.

Fluorescence images were acquired with a LSM 510 Meta laser scanning confocal microscope (Carl Zeiss, Welwyn Garden City, UK). All fluorescence images were analyzed using Zeiss LSM image browser software.

Results and Discussion

Photochemical Oxidation of Surface Bound Thiol Groups

The UV induced oxidation of surface bound thiol groups to sulfonates was first described by Bhatia and co-workers.[27] Based on these findings, a detailed investigation on the photo-oxidation reaction of a MPMS layer, i.e. the reaction kinetics of sulfonic acid formation and its influence on the surface polarity, was performed by XPS, SIMS and contact angle measurements (presented in the supporting information) revealing a maximum yield after 20 min (44.4 J/cm^2) of UV-illumination.

In Figure 2 (left), the S2s region of the XPS-analysis is shown for the MPMS layer before and after UV illumination (E=44.4 J/cm^2) under ambient conditions. In the spectrum of the as-prepared film the signal at 228 eV is typical for a mercapto group. UV irradiation leads to a decrease in the intensity of the thiol peak, while a new peak is observed at 233 eV. This signal can be attributed to sulfonic acid groups, which is in accordance to the findings of Bhatia et al.. Figure 2 (right) shows the variations of the signal intensity in the S2s region at both 228 eV and 233 eV as a function of UV exposure time. It was found that the SH species decreased rapidly in intensity following exposure to UV-light, whereas an increase in the sulfonate signal with a similar rate was observed. Both signals reached a limiting value after an illumination time of 20 min (44.4 J/cm^2).

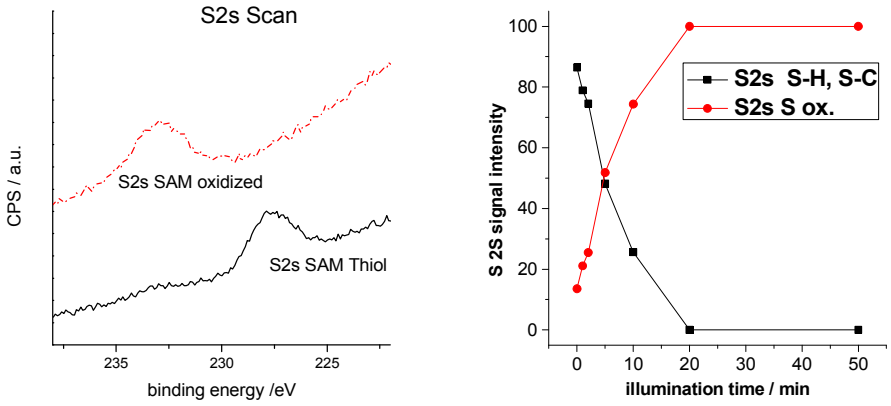

Figure 2: left: XPS spectra of the sulfur 2s signal before (solid line) and after (dotted line) UV-illumination. Right: variations of the signal intensity in the S2s region at both 228 eV (S-H, S-C) and 233 eV (SO_3H) as function of UV exposure time.

Using this photochemical approach, micro-patterned functionalized surfaces were prepared by contact lithography with appropriate chromium-quartz glass masks. Such samples were analyzed using SIMS imaging to confirm that photooxidation was occurring. Figure 3 shows the representative negative polarity SIMS images of a single region. The image on the left shows combining images of the ions corresponding to the unoxidized thiol groups (i.e. the S^- and the HS^- ions) and the right image shows the combined images of the ions corresponding to the oxidized thiol (i.e. the SO_2 and SO_3 ions). The left image shows bright regions in the areas that were protected by the mask (i.e. unexposed regions), and dark regions in the areas that are exposed to UV. This corresponds to a higher density of unoxidized thiol species in the unexposed regions. The right image shows the opposite contrast to the left image, where the bright regions are the areas of the sample that has been exposed to UV and the dark regions are the areas that were covered by the mask. This corresponds to a higher density of SO_2 and SO_3 groups in the areas of the sample that are exposed to UV. This is consistent with the contact angle, XPS and AFM results and also with previous studies of the photooxidation of alkanethiols on metal substrates.

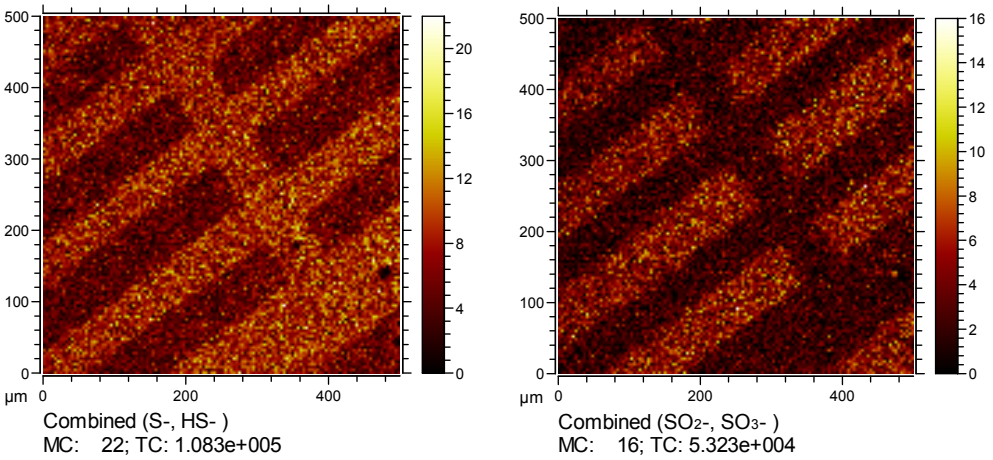

Figure 3: Negative polarity SIMS images of photooxidation patterns of a thiol surface, showing left: combined images of S- and HS- ions. Right: combined images of SO_2 and SO_3 ion images.

To reveal a material contrast between illuminated and non-illuminated regions of the patterned samples, additionally, friction force microscopy (FFM) was performed. In this mode a soft cantilever is scanned perpendicular to its long axis. Lateral forces resulting from the interaction of the tip with the substrate lead to a twist of the cantilever depending on the friction of the surface. A representative FFM image of a patterned surface is shown in Figure 4 (left). The exposed regions, in which the mercapto groups have undergone the photo-induced oxidation reaction, yield bright contrast (high friction), while the masked areas exhibit darker contrast.

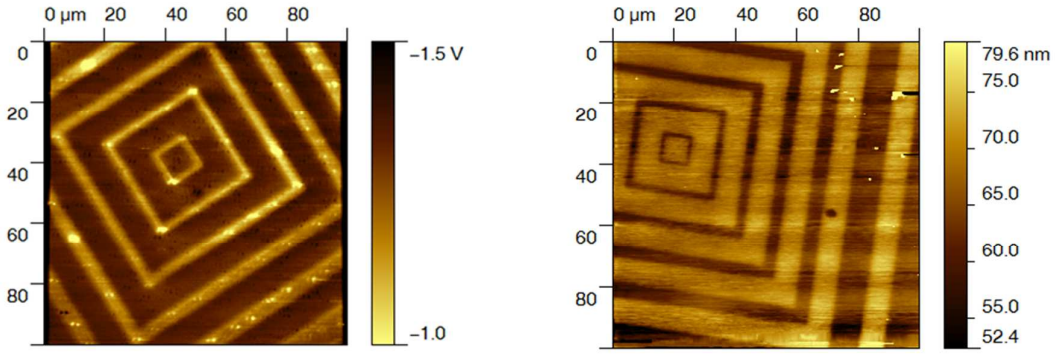

Figure 4: left: Friction force images after patterned UV-illumination. Bright contrast indicates high friction force (the SO_3H-terminated regions), while dark contrast indicates low friction (S-H terminated regions). Right: Atomic force microscopy image of pre-patterned MPMS layer after surface induced polymerization.

Surface Induced Polymer Brush Formation

It has been widely reported that surface-bound thiyl radicals can initiate a polymerization reaction in presence of (meth)acrylate monomers resulting in the formation of polymer brushes under inert conditions.[31,32,33] We examined the possibility of carrying out patterned brush growth by exposing a film of monomer, covering a pre-structured thiolated surface, to UV illumination. This means attempting to carry out the polymerization in air. Astonishingly, this approach leads also to polymer brush structures with well-defined dimensions, being uniform in all three spatial directions as shown in Figure 4 (right).

The cross section of the topography is added in the supporting info. In this experiment, neat oligoethyleneglycol methacrylate (OEGMA) was deposited on the pre-patterned MPMS layers (Figure 4, left) and covered by quartz glass, before being exposed to UV irradiation. As displayed in Figure 4 (right) the intact thiol moieties initiated a polymerization reaction in presence of OEGMA, while the sulfonate groups remained inactive. Alternatively, polymer brush structures have been obtained by patterned illumination using neat OEGMA on pristine MPMS layers covered by a mask. Figure 5 shows AFM data for micropatterned structures formed in this way. Polymer structures grew selectively in the irradiated areas. Studies of individual samples at different UV irradiation times (0.5-6 minutes) revealed that a minimum dose of 2 J/cm^2 is required in order to obtain well-defined brushes. Using the threshold dose of 2 J/cm^2, we observed that the monomer solution between the substrate and mask became highly viscous. This indicates significant polymer content in the monomer phase due to auto-initiated polymerization of methacrylate in solution under UV irradiation.

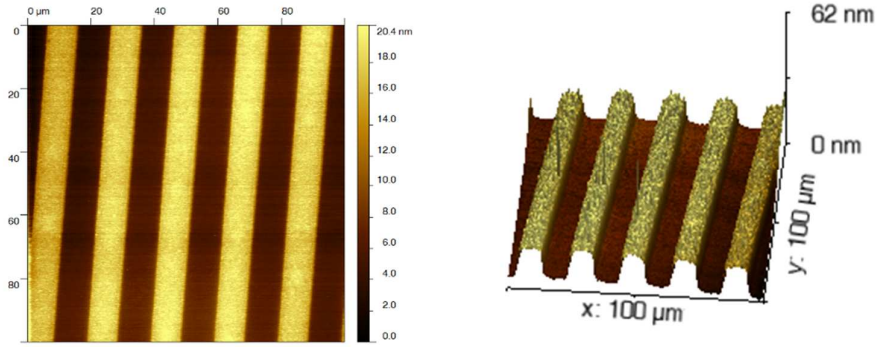

Figure 5: Atomic force microscopy image (left) and three dimensionally reconstructed image of the obtained polymer brushes (right).

Polymerization also occurred when the sample was immersed in a solution of OEGMA in 1,4-Dioxane. The concentration of OEGMA in the used monomer solution influenced significantly the height of the obtained polymer brushes. Using a 100 µm line mask and a illumination dose of 5.3 J/cm^2 it was possible to exercise some control over the growth of the polymer brushes by variation of the OEGMA content in 1,4-Dioxane, under ambient conditions, as shown in Figure 6 (left). The thickness of the grown brush structures increases monotonically with the concentration of monomer in the range 40% to 90%.

In order to exclude a "grafting to" mechanism, which could be based on a coupling of self-polymerized macroradicals with surface bound thiyl radicals, control experiments were performed. Solutions with 50 vol%, 70 vol% and 100 vol% OEGMA in 1,4-Dioxane were deposited on non-modified Si-wafer and were subsequently covered with a glass slide. UV illumination (E=5 J/cm^{-2}) also leads to an increase in viscosity of the monomer solution as observed in the previously described patterning experiments. GPC analysis, however, revealed only little variation in the molecular weight of the formed macromolecules (M_n is in the range between 8300 and 9300 g/mol) in all three different solutions (see supporting info). As there are significant changes in the brush heights using these concentrations, the brush growth is not explainable with a "grafting to" mechanism, because in this case different molar weights would be expected, relating to the observed height variations.

The influence of the polymerizable group on the brush formation was also investigated. The corresponding acrylate monomer, oligoethyleneglycol acrylate, was used for brush growth (E= 1.75 J/cm^2). It was found that the thiyl initiated polymerization of acrylates led to a film thickness of several microns as presented in Figure 6 (right). This fact can be explained by the significantly higher rate of polymerization, i.e. reactivity, of acrylates in solution compared to methacrylates.

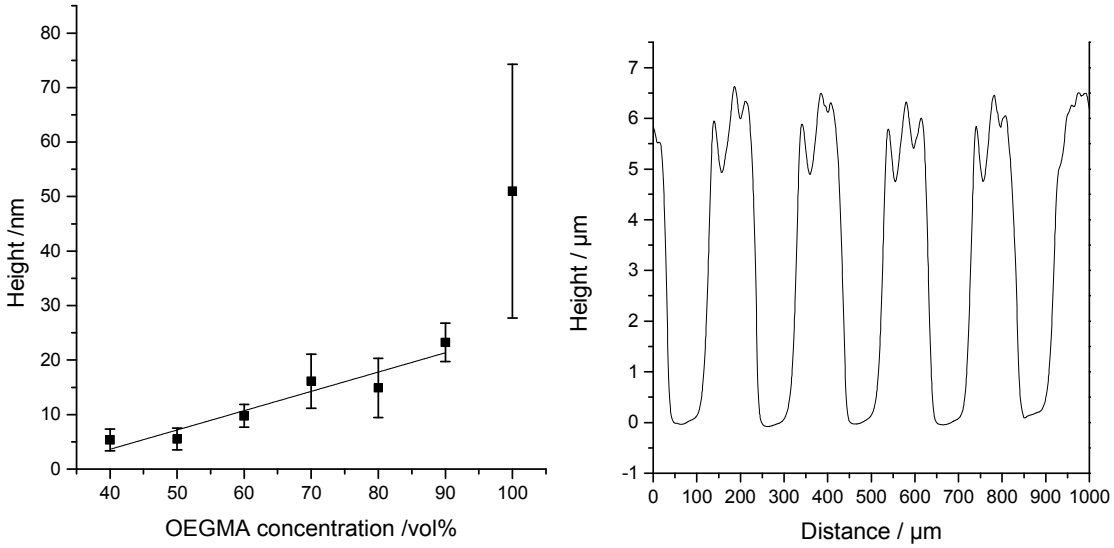

Figure 6: Height of the polymer brushes as a function of OEGMA concentration (left) and surface profile of the corresponding acrylate (OEGA) brushes

Derivatization of Sulfonic Acid Groups

In order to expand the toolbox of reactions for surface modification together with the aforementioned oxidation of mercapto groups we have evaluated a direct activation of the sulfonic acid groups by triphenylphosphine ditriflate (TPPDF). This activation procedure has previously been reported by Caddick et al. for the synthesis of biologically active sulfonamides.[34] The reaction scheme is shown in Figure 7 (left). Following photooxidation to give the sulfonate, sample surfaces were treated with pyridine in water after which they were immersed in a solution of triphenylphosphine ditriflate in dichloromethane a solution of heptadecafluoroundecylamine (HDFA) and TEA in dichloromethane was added after 30 min. The reaction between the activated sulfonic acid group

and the amine was expected to yield a sulfonamide bond. The fluorinated reagent was selected because it would yield characteristic peaks in XPS spectra if reaction occurred. XPS C1s spectra exhibited additional CF_2 (292.0 eV) and CF_3 (293.8 eV) peaks as shown in Figure 7 (right), confirming that reaction had indeed taken place. Table 1 shows the peak assignments.

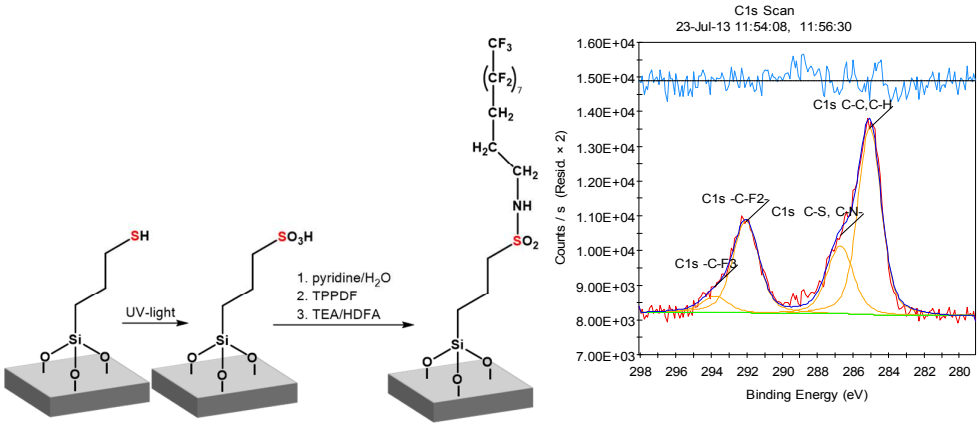

Figure 7: left: Scheme of the photo-oxidation and subsequent chemical modification. Right: XPS spectra of the carbon 1s signal after derivatization of the formed sulfonic acid groups with HDFA.

Table 1: Peaks fitted to the C1s high-resolution XPS spectra

functional groups	binding energy (ev)
Si-C	283.5
C-C	285.0
$C-F_2$	292.0
$C-F_3$	293.8
C-N, C-S	286.7

The peak at 286.7 is composed of signals from the C-N bond of the formed sulfonamide group and from the C-S bond, respectively. The wide scan (see supporting info) revealed elemental intensity ratios C:F of 1:1 which suggests a derivatization yield of 50%. The incomplete derivatization of the molecules is probably due to steric hindrance of this long chain fluorocarbon. However, perfluorinated organic molecules are also highly susceptible to X-ray-induced damage, and this may

contribute to a reduction in the fluorine C1s signal of the wide scan spectrum. Besides XPS the derivatized surfaces were also characterized by means of contact angle measurements. The conversion of the photogenerated sulfonic acid groups to sulfonamides bearing a fluoroalkyl chain results in a significant increase of the static contact angle of water from 18° to 81°. The obtained value of 81° is lower than other studies have revealed for fluoroalkyl derivatization of SAMs.[35] This fact can be explained by the non-quantitative conversion of the highly polar sulfonic acid groups.

Protein Patterning

Protein patterning was carried out by combining the thiol induced polymerization reaction with the derivatization of the photo-generated sulfonates. First, the sample was photopatterned to create sulfonate regions surrounded from masked areas in which the thiol groups remained intact. Subsequently, polymer brushes were grown by a surface induced polymerization initiated by thiyl radicals. In the final step, NTA derivatives were coupled to the sulfonate functionalized areas using TPPDF activation, to facilitate selective immobilization of His-tagged GFP.

Figure 8 shows samples patterned in this way. MPMS films were patterned using both mask-based and interferometric exposure. OEGMA solution was deposited onto the samples, and they were illuminated with UV light, yielding polymer brushes in the non-oxidized areas. The oxidized areas were functionalized by NTA molecules attached to amino-terminated linkers using TPPDF activation. Highly fluorescent GFP was coupled to the patterns via the specific interaction between Ni-NTA and His-tagged proteins, and the samples were imaged using fluorescence microscopy (Figure 8). Bright fluorescence was observed from the oxidized and derivatized areas, whereas the OEGMA brushes remain dark.

In control experiments, pre-oxidized samples were immersed directly in GFP solutions after OEGMA brush formation. In this case, only a very poor coverage of GFP was observed and therefore dark images were obtained. This finding can be explained by the antifouling properties of (1) the formed

OEGMA brushes and (2) of the sulfonate groups in the oxidized areas as reported by Bhatia et al.[27] Immersion of patterned samples in a solution of imidazole (100 mM of imidazole in water for 4 hours) also led to the disappearance of fluorescence, indicative of displacement of the His-tagged protein by imidazole, evidence for site-specific immobilization in the patterned regions (squares) in Figure 8(b).

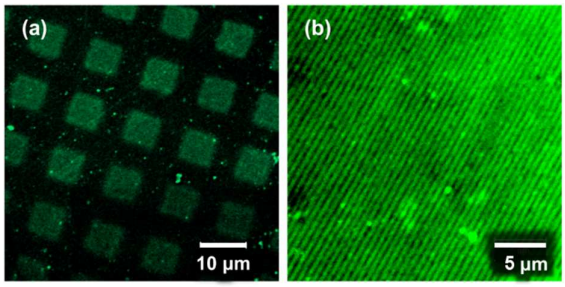

Figure 8: Fluorescence micrograph images of patterned MPMS layer (a) contact lithography; (b) interference lithography) after brush growth and subsequent GFP immobilization.

Conclusion

In the present work, surface bound thiol groups have been investigated for the realization of complex bionanostructures. Consequently, mercaptosilane films were patterned by utilizing UV-induced photo-oxidation of the thiol to yield sulfonate groups. Micrometer-scale patterns were formed in a mask-based process, and nanopatterns were formed by interferometric lithography (IL) using a Llyod's mirror dual-beam interferometer. The sulfonate was used for a selective immobilization of amino-functionalized molecules after activation with triphenylphosphine ditriflate. Moreover, protein-resistant polymer brushes containing oligoethyleneglycol groups were grown from the intact thiol groups by a surface-induced polymerization reaction. Depending on both the concentration and the choice of the used polymerizable group, i.e. methacrylate or acrylate, brush heights between several nano (methacrylates) and micrometres (acrylates) could be realized.

By the combination of both reactions, it is possible to couple amino-labelled nitrilotriacetic acid (NH_2-NTA) to sulfonate-functionalized regions, facilitating the site-specific binding of green fluorescence protein (GFP) and to realize protein-resistant polymer brushes by exploiting remaining thiol groups. Control experiments revealed the antifouling behaviour of the obtained POEGMA brushes as well as the immobilization of the GFP via Ni-NTA and His interaction. IL exposure also yielded photooxidation of mercapto groups and facilitated nano patterning.

The versatility of thiol groups paves the way towards novel strategies for surface conjugation enabling the fabrication of complex protein nanopatterns beyond thiol-ene chemistry.

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TOC:

Versatile Thiol-Based Reactions for Micrometer- and Nanometer-Scale Photopatterning of Polymers and Biomolecules

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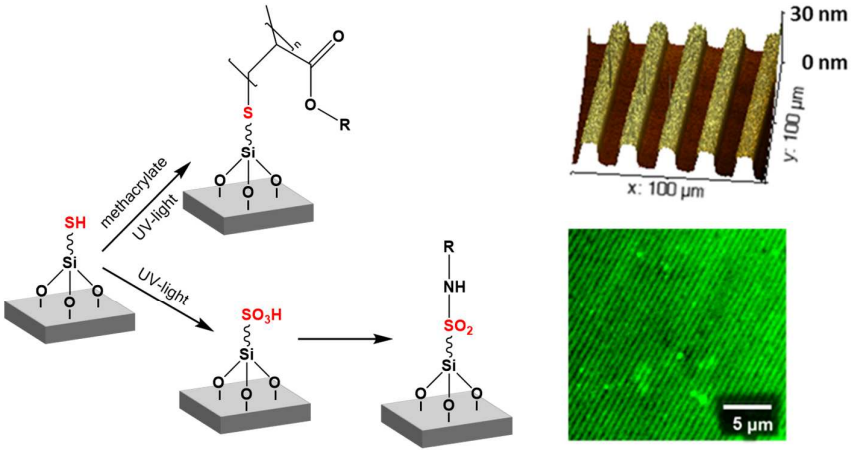

Thiol-based reactions were applied to enable the photochemical patterning of polymer brushes and green fluorescent protein on silicon oxide surfaces.